BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

We claim:

1. A method for regeneration of cotton via somatic embryogenesis with substantially synchronized development of embryos after short duration inositol starvation, said process comprising the steps of :

(i) cutting from the germinated cotton seedling the explant, selected from a group consisting of cotyledon , hypocotyl ,mesocotyl and mixtures thereof;

(ii) culturing the explant for the purpose of callus induction in a first solid medium, in a culture medium containing glucose as the carbon source supplemented with Gamborg B5 vitamins , 2,4-D and BA and inositol, at a temperature between 23 to 33°C in light intensity of at least 90 μ mol/m^2/s under a 16 hour photopeiod for a period of 3-5 weeks , to enable dedifferentiated callus to form from the explant;

(iii) transferring the callus from the first solid callus induction medium to a liquid medium comprising a basal medium containing glucose as the carbon source and supplemented with Gamborg B5 vitamins and culturing the suspension generated thereof at a temperature from 23 to 33° C in a reduced light intensity of 20-40 μ mol/m^2/s, under a 16 hour photoperiod for a period of time sufficient to form embryogenic clumps;

(iv) screening the cell suspension through metal sieves of different pore sizes to select embryogenic cells and/or clumps and subculturing the embryogenic callus containing somatic embryos to said basal medium;

(v) subjecting the embryogenic mass / clumps to inositol deprivation ,consequent upon subculturing it to said basal medium devoid of inositol for a sufficient period of time and then returning the culture to inositol containing medium to enable the somatic embryos to synchronize developmentally;

(vi) transferring bipolar somatic embryos to an embryo germination medium on a support and growing the embryos in embryo germination medium upto the plantlet stage developed sufficiently for transfer to soil;

(vii) further transferring the plantlets to a potting mix for acclimatization and then to field;

2. A method as claimed in claim 1 wherein the explants are derived from cotton or any other plant seedlings.

3. The method as recited in claim 1, wherein the explant is derived from cotton cv Coker 312 and the seedlings are developed by

(i) sterilizing cotton seed in a sterilization solution of 0.1% $HgCl_2$ for 5-10 min. preferably 7 min.,

(ii) rinsing the seed in sterile water 4-6 times ,

(iii) scorching the seed in flame of a spirit burner for 5-10 seconds ,

(iv) inoculating the seed on seed germination medium ,

(v) growing the seed in the seed germination medium in light or dark at a temperature of 23 degree to 33 degree C for a period of 6-12 days , preferably 9-10 days,

(vi) excising the explant from the seedling.

4. A method as claimed in claim 3 wherein seed germination medium is a liquid medium comprising salts of Murashige and Skoog and Gamborg B5 vitamins at half of its concentration .

5. A method as claimed in claim 3 wherein carbon source in seed germination medium is selected from a group consisting of sucrose and glucose at a range of 1 to 3% wt./ vol.

6. A method as claimed in claim 1 wherein said first solid callus induction medium comprises following components of Murashige and Skoog medium:

Component	Conc. (mg/L)
a. Salts of Murashige and Skoog (1962) medium:-	
NH_4NO_3	1650
KNO_3	1900
$CaCl_2.2H_2O$	440
$MgSO_4.7H_2O$	370
KH_2PO_4	170
KI	0.83
H_3BO_3	6.2
$MnSO_4.4H_2O$	22.3
$ZnSO_4.7H_2O$	8.6
$Na_2MoO_4.2H_2O$	0.25
$CuSO_4.5H_2O$	0.025
$CoCl_2.6H_2O$	0.025
Na_2.EDTA	37.3
$FeSO_4.7H_2O$	27.8
b. Organics	
Myo-inositol	100

7. A method as claimed in claim 1, wherein Gamborg B5 vitamins , wherever included comprise:

Component	Conc. (mg/L)
Nicotinic Acid	1.0
Pyridoxine HCl	1.0
Thiamine HCl	10

8. A method as claimed in claim 1, wherein 2,4-D as exogenously supplied auxin in first solid callus induction medium is selected from a range of 0.44 to 4.4 μM , preferably 1.76 to 2.64 μM.

9. A method as claimed in claim 1, wherein BA as exogenously supplied cytokinin in first solid callus induction medium is selected from a range of 0.22μM to 2.2μM, preferably 0.66μM to 1.00μM.

10. A method as claimed in claim 1, wherein gelling agent in said first solid callus induction medium is selected from a group consisting of agar in the range of 0.6-0.8% wt./vol., preferably 0.7% and phytagel in the range of 0.15-0.29% wt./vol., preferably 0.22% wt./vol..

11. A method as claimed in claim 1, wherein said first solid callus induction medium contains glucose as the primary carbon source.

12. A method as claimed in claim 1, wherein said explants are cultured on said callus induction medium at a temperature between 23 to 33°C, preferably between 27 to 29°C in light intensity of at least 90 μmol/m^2/s under a 16 h photoperiod for period of not more than of 3-5 weeks, to enable dedifferentiated callus to form from any of the said explant.

13. A method as claimed in claim 1, essentially including the step of transferring callus from the said first solid callus induction medium to a liquid medium in Ehrlenmeyer flasks at a packing density of 600 to 1000 mg of callus/50 ml of media preferably, 800 mg/50 ml and shaking the culture in this and all subsequent steps until somatic embryos are taken out for germination on a gyratory shaker at 110-130 rpm.

14. A method as claimed in claims 1 and 13, wherein said embryogenesis induction medium is a basal liquid medium comprising M&S salts, Gamborg B5 vitamins, inositol and glucose as the carbon source.

15. A method as claimed in claims 1 and 13, wherein plant cell suspension embryogenic mass and somatic embryos generated thereof in liquid medium are

incubated at a temperature from 23 to 33°C, preferably 27-29°C in light intensity of 20-40 μmol/m^2/s, typically 27-33 μmol/m^2/s under a 16 h photoperiod.

16. A method as claimed in claim 1, wherein said embryogenic mass/clumps are subjected to inositol deprivation for a period of 8 to 12 days, preferably, 10 days in inositol deprivation medium comprising MS basal salts, Gamborg B5 vitamins, glucose as carbon source but no inositol, leading to developmental synchronization of somatic embryos.

17. A method as claimed in claim 1, wherein said first solid callus induction medium has a pH in the range of 5.4-6.2 and the entire liquid media in said process has a pH in the range of 5.2 – 5.8, being sterile as a result of autoclaving at 121°C, 16 psi for 16 minutes.

18. A method as claimed in claim 1, wherein potting mix comprises of garden soil: sand : Peat moss: vermiculite typically in 2:1:1:1 ratio.

19. A method as claimed in claim 1, wherein developmental synchrony of somatic embryogenesis is utilized for multiplication of elite cotton cultivar or development of transgenic cotton cultivar.

20. A method as claimed in claim 1, wherein the inositol depletion is applied to plant species other than cotton for enhancing embryogenesis in tissue culture.

21. A method as claimed in claim 1 wherein said culture medium and basal medium comprise of Murashige and Skoog medium.

22. A method as claimed in claim 1 wherein said period of time sufficient to from embryonic clumps comprises 12-32 days.

23. A method as claimed in claim 1 wherein said subculturing the embryogenic callus containing somatic embryos to said basal medium is carried out at intervals of 8-12 days.

24. A method as claimed in any preceding claim wherein said embryogenic mass/clumps are subjected to inositol deprivation for a period of 8 to 12 days, preferably, 10 days.

25. A method as claimed in claim 1 wherein said support for said embryo germination medium comprises vermiculite.